Available online at www.sciencedirect.com
ScienceDirect
Industrial Crops and Products 25 (2007) 136–146
INDUSTRIAL CROPS AND PRODUCTS
AN INTERNATIONAL JOURNAL
www.elsevier.com/locate/indcrop

Optimization for enzyme-retting of flax with pectate lyase

Danny E. Akin [a,*], Brian Condon [b], Miryeong Sohn [a], Jonn A. Foulk [c], Roy B. Dodd [d], Luanne L. Rigsby [a]

[a] *Russell Research Center, ARS-USDA, PO Box 5677, Athens, GA 30604, USA*
[b] *Southern Regional Research Center, ARS-USDA, New Orleans, LA 70179, USA*
[c] *Cotton Quality Research Station, ARS-USDA, Clemson, SC 29633, USA*
[d] *Department of Agricultural and Biological Engineering, Clemson University, Clemson, SC 29634, USA*

Received 24 April 2006; accepted 7 August 2006

Abstract

Flax (*Linum usitatissimum* L.) is an important commercial crop that supplies both linseed and bast fibers for multiple applications. Retting, which is a microbial process, separates industrially useful bast fibers from non-fiber stem tissues. While several methods (i.e., water- and dew-retting) are used to ret flax, more recently enzymes have been evaluated to replace methods used currently. Alkaline pectate lyase (PL) from the commercial product BioPrep 3000 and ethylenediaminetetraacetic acid (EDTA) from Mayoquest 200 as a calcium chelator were used in various formulations to ret flax stems. Retted stems were then mechanically cleaned through the USDA Flax Fiber Pilot Plant and passed through the Shirley Analyzer. The PL and chelator effectively retted flax from both fiber flax and linseed stems, and the use of enzyme plus chelator retted flax stems better than either component alone. Fiber yield and strength were greater than retting with a mixed-enzyme product that contained cellulases. Retting with PL and chelator was optimized based on fine-fiber yield, remaining shive content, and fiber properties. PL at levels of about 2% of the commercial product for 1 h at 55 °C followed by treatment with 18 mM EDTA for 23–24 h at 55 °C provided the best fibers based on these criteria. Yield and fiber properties determined by these tests were not improved with PL levels of 5% of the commercial product.
© 2006 Published by Elsevier B.V.

Keywords: Pectin; Chelator; Shive content; Strength; Fineness

1. Introduction

Flax (*Linum usitatissimum* L.) is the source of linen and has provided high-quality fibers for textiles for thousands of years (Sharma and Van Sumere, 1992a). Specialty papers are often made with flax fibers as they provide strength for thinner papers. Natural fibers have been promoted for composites and other industrial uses, particularly automotive parts (Lepsch and Horal, 1998).

Flax fibers are formed in the cortical regions of stems (Van Sumere, 1992). To extract fibers for industrial use, stems are retted, which is usually a microbial process that separates fiber from non-fiber stem tissues. For high quality linen from western Europe that requires a long fiber with special processing, water-retting was the method of choice for many years. In the mid-1990s water-retting was discontinued because of the extensive stench and pollution arising from anaerobic bacterial fermentation of the plant materials, high costs, and a putrid odor of the resulting fibers (Sharma, 1987a; Van Sumere,

* Corresponding author. Tel.: +1 706 546 3482; fax: +1 706 546 3607.
E-mail address: deakin@qaru.ars.usda.gov (D.E. Akin).

0926-6690/$ – see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.indcrop.2006.08.003

1992). Dew-retting is now the primary process used for the industrial production of flax fibers (Van Sumere, 1992) although water retting is still carried out in some places (Daenekindt, 2004). Despite the widespread use of dew-retting to obtain commercial fibers from flax, disadvantages also exist for this method that include: low and inconsistent quality, restriction to certain climatic regions, occupation of land for several weeks during retting, and a product contaminated with soil. As a result of these problems, a better flax retting method has been sought for some time (Van Sumere, 1992; Sharma and Faughey, 1999).

In retting, microbial activity (e.g., anaerobic bacteria in water-retting or aerobic fungi in dew-retting) causes a partial degradation of the components that bind tissues together, thereby separating the cellulosic fibers from non-fiber tissues. Earlier work has clearly indicated the requirement of pectinases in flax retting (Sharma, 1987a; Van Sumere, 1992). Enzyme-retting has been evaluated as a replacement for current retting methods (Sharma and Van Sumere, 1992b; Van Sumere, 1992). Products with various enzymes, such as polygalacturonases, pectin lyase, hemicellulases, and cellulases have been screened for retting (Sharma and Van Sumere, 1992b). Flaxzyme, a mixed-enzyme commercial product of Novo Nordisk from *Aspergillus* sp., was developed earlier and was reported to produce fibers with good yield and quality (Van Sumere and Sharma, 1991). The yield and tactile qualities of enzyme-retted fibers were similar to that from high-quality water-retted fibers. A disadvantage, however, was the potential lower fiber strength due to the continued activity of the cellulases in the mixtures. Treatment with an oxidizing agent, such as sodium hypochlorite, or reagents giving a high pH was applied to denature the enzymes and prevent the continuing cellulolytic activity. Research on enzyme-retting led to a series of patents and to a semi-industrial scale trial (Van Sumere, 1992), but no commercial system was developed. Flaxzyme by this name is no longer available.

Recent research has explored the use of other pectinase-containing products for retting bast fiber plants, including flax (Brühlmann et al., 2000; Akin et al., 2004; Antonov et al., 2005; Kozlowski et al., 2005). In our work (Akin et al., 2004), the objective is the economic production of short staple fibers for blending with cotton or other fibers and for industrial applications such as composites. The requirements to maintain long fiber length and other restrictions necessary for production of traditional linen are unnecessary, and new methods can be explored to produce a total fiber product from diverse sources of flax. Results from several studies (Akin et al., 2004) have indicated that Viscozyme L with ethylenediaminetetraacetic acid (EDTA) effectively retted flax. This enzyme in combination with EDTA was used in a spray enzyme-retting method (Akin et al., 2000) or by brief (2 min) soaking until stems were saturated. Fibers produced from various formulations and flax sources were evaluated and ranked in test yarns (Akin et al., 2001). Use of Viscozyme and EDTA became the basis on which other products and protocols were compared within our laboratory. Although washing the retted fibers prevented continued and undesired enzyme activity, use of high levels or long incubation times with Viscozyme weakened the fibers (Akin et al., 2004). We, therefore, investigated other strategies to maintain fiber strength with enzyme-retting. In work recently carried out on new commercial products for retting, alkaline pectate lyase was shown to effectively ret flax and maintain fiber strength (Akin et al., unpublished). The objective of the present study is to optimize the formulation of pectate lyase and chelator from commercial products for retting a variety of fiber flax and linseed samples and to define the characteristics of the processed fibers.

2. Materials and methods

2.1. Chemicals

Mayoquest 200, a commercial product with about 38% EDTA (Lynx Chemical Group, L.L.C., Dalton, GA) was used a chelator at concentrations of 18 mM EDTA unless otherwise indicated. Viscozyme L from Novozymes North America, Inc. (Franklinton, NC) is a multienzyme commercial product marketed for breaking apart of plant materials. This enzyme complex has been used for retting flax, and its formulations were the basis for comparing other methods (Akin et al., 2004). BioPrep 3000L is a liquid commercial alkaline pectate lyase (PL) produced by a genetically modified *Bacillus lichiniformis* with a reported activity of 3000 alkaline pectinase standard units (APSU)/g. BioPrep was developed by Novozymes North America, Inc. and is marketed by Dexter Chemical LLC (Bronx, NY) under the tradename Dextrol Bioscour 3000.

2.2. Flax samples

Different varieties of flax plants grown primarily for either fiber or for linseed was evaluated. ‘Ariane’ flax that which produces a high quality fiber (Brown et al., 1986) was grown as a winter crop in the coastal plains of South Carolina, USA, in 1998–1999 and harvested after full seed maturity. This cultivar was produced in a 3-ha field experiment and was drum-mowed, dried, baled,

and stored without further weathering. 'Jordon' flax was grown at the Pee Dee Research and Education Center, Florence, SC, in the winter of 1999–2000 and harvested as described for Ariane, but before full seed maturity for high quality fiber. 'Omega', 'York', and 'Hermes', which are oilseed flax varieties, were grown in test plots to full seed maturity in the summer of 2004 at the Carrington Research Extension Center, North Dakota State University. The Omega and York samples received rain and underwent considerable weathering after harvest. Hermes, however, did not receive rain and appeared bright and unweathered before the tests.

2.3. Enzyme-retting and processing of stems

Flax stems were crimped through either fluted rollers (Akin et al., 2000) or the nine-roller calender of the USDA Flax Fiber Pilot Plant (Akin et al., 2005). For retting, 150-g samples of crimped stems were briefly soaked (2 min) in enzyme solutions, drained for 30 s, and incubated in conditions optimal for enzyme activity. For Viscozyme and some tests with PL, the enzymes and chelator were combined and used as one formulation at pH and temperature optimal for the enzyme (i.e., pH 5 and 40 °C for Viscozyme and pH 9 and 55 °C for PL). For the other PL tests, crimped stems were soaked 2 min in enzyme buffered with sodium borate (pH 8–9) and incubated in plastic bags at about 55 °C. After incubation for specified times, the flax stems were removed from the incubator, again soaked (without washing) in chelator solution for 2 min and drained as described. The flax stems were then placed back in plastic bags in the incubator at about 55 °C for selected incubation times. After retting, the flax straw was washed for 2 min in running water and air dried. All treatments were carried out in triplicate 150-g samples.

The enzyme-retted, washed, and dried stems were processed through the USDA Flax Fiber Pilot Plant (Flax PP) (Akin et al., 2005) in the following order: nine-roller calender 1×, top shaker 1×, scutching wheel 1×, five-roller calender 1×, top shaker 2×. After processing through the Flax PP, fiber samples were evaluated for shive content with a near infrared reflectance spectroscopy (NIR) (Sohn et al., 2004) using a recently developed test method (ASTM D 7076-05, 2005). Samples were then conditioned at 21 °C and 65% relative humidity and passed through the Shirley Analyzer (SDL America, Charlottsville, NC, USA) to separate the fine fiber (i.e., Shirley-cleaned fiber). In some studies, the waste fiber plus shive that was not collected as fine fiber after passing through the Shirley Analyzer was passed through the system for several times, and the fine-fiber yield was determined from the sum of these multiple passes.

2.4. Fiber tests

Pilot plant-cleaned and Shirley-cleaned fiber both were used to assess retting efficiency, based on the fiber yield and % shive content as criteria. Shirley-cleaned fibers were then subjected to a series of tests to determine the fiber properties. Shirley-cleaned fibers were assessed for % shive content by the NIR method (ASTM D 7076-05, 2005) with triplicate readings for each of three replicated enzyme treatments. The strength in g/tex and the % elongation for fiber bundles at 6.25 mm gauge distance were determined for six trials for each replicate with the Stelometer as in the cotton system (ASTM D 1445-95, 2003). Fineness was determined using airflow (Akin et al., 1999) and a formula to give specific surface index based on a series of graded fiber widths (ASTM D 7025-04a, 2005) for 5-g duplicate samples for each replicate. The tactile softness of a series of enzyme-retted samples was ranked by two scientists experienced in handling flax and cotton fibers from 1 (softest) to 10 (roughest).

Test yarns from the 1:1 blend proportions (initial proportions) of cotton and enzyme-retted, Shirley-cleaned flax fibers were prepared on a mini-spinning system and tested using standard methods (Akin et al., 2001). Waste at various processing stages was collected and weighed to determine fiber losses.

2.5. Scanning electron microscopy

Intact flax stem segments about 2 mm long from the central position of one stem were left unretted or enzyme-retted in a similar manner to other samples with PL, PL followed by chelator, or chelator alone. Stem segments were fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer at 4 °C for 48 h. Samples were rinsed 3× with buffer and air-dried. In other studies, fibers from 150-g retting studies were used without fixation. Stem segments and fibers were mounted on carbon SEM stubs, sputter-coated with gold, and viewed with a JEOL JSM 5800 scanning electron microscope at 15 kV.

3. Results

Earlier work had shown that PL produced fibers with good properties and with a higher tenacity than Viscozyme. Based on general recommendations for bioscouring cotton with PL from BioPrep (personal communication, S. Salmon, Novozymes North America,

Table 1
Fiber yield and properties from flax enzyme-retted with pectase lyase and chelator

Enzyme formulation[a]	Uptake (ml)	Fiber yield (% straw)[b]		Shive content in fiber (%)[c]		Strength (g/tex)	Fineness (airflow)
		Flax-PP	Shirley cleaned	Flax-PP	Shirley cleaned		
PL only	278	38.9 ± 0.9 a	6.0 ± 1.2 b	19.8 ± 1.7 a	4.1 ± 0.2 a	33.2 ± 2.4 bc	3.8 ± 0.1 b
PL; C[d]	408	36.9 ± 2.3 ab	9.8 ± 1.0 a	18.9 ± 1.8 a	3.6 ± 0.6 a	35.7 ± 2.2 ab	2.7 ± 0 d
PL + C[e]	291	36.7 ± 3.5 ab	7.7 ± 1.5 ab	18.0 ± 3.5 a	3.7 ± 1.0 a	34.9 ± 2.0 b	3.0 ± 0.6 d
PL + B + Cl[f]	326	34.3 ± 1.8 bc	5.8 ± 1.3 b	14.5 ± 5.7 a	3.2 ± 1.1 a	34.8 ± 4.8 b	3.6 ± 0.5 bc
PL + B + Cl[g]	340	34.0 ± 0.6 bc	5.8 ± 1.2 b	16.6 ± 2.0 a	4.1 ± 1.6 a	32.6 ± 1.3 bc	3.0 ± 0.3 cd
0.05% Viscozyme + C	305	30.7 ± 2.4 bc	3.4 ± 0.4 c	19.4 ± 3.0 a	4.6 ± 1.3 a	27.4 ± 6.0 c	3.9 ± 0.3 b
Untreated	–	36.1 ± 4.5 ab	3.2 ± 1.5 c	19.6 ± 3.8 a	6.2 ± 1.0 a	42.0 ± 5.5 a	5.2 ± 0 a

Values followed by different letters (a–c) within columns and different tests differ at $P \leq 0.05$.

[a] Enzymes and chemicals are used as provided by suppliers. PL: pectate lyase in 0.1% BioPrep. C: chelator as Mayoquest 200 added to provide 18 mM EDTA, based on a 38% EDTA solution. pH's were adjusted to recommendation of suppliers. Flax was mature Ariane was grown in South Carolina during the winter of 1998–1999 and crimped through fluted rollers.

[b] Yield from stems cleaned through the USDA Flax Fiber Pilot Plant (Flax-PP) and from fiber subsequently passed through the Shirley Analyzer.

[c] Determined by near infrared spectroscopy.

[d] PL;C indicates sequential of enzyme followed chelator.

[e] PL + C indicates both components combined in one solution.

[f] PL + B + Cl-enzyme in 0.5 mM sodium borate, 1.83% Barapon and 0.15% Clavodene.

[g] PL + B + Cl-enzyme in 25 mM sodium borate, 1.83% Barapon and 0.15% Clavodene.

Inc.), a series of evaluations was carried out to optimize the use of PL for retting flax. Several formulations using PL in 0.1% (v/v) of the commercial product were used in our pilot plant procedure for enzyme-retting and cleaning fiber (Table 1). Results were compared with Viscozyme plus chelator and with no retting. The recommendation for bioscouring cotton was to treat with PL about 15 min prior to adding chelators (S. Salmon, personal communication). In our evaluation (Table 1), a sequential treatment with PL followed by chelator tended to give fibers with slightly higher yields and better properties than a combined formulation, but differences were not significant ($P > 0.05$). This trend suggests some deactivation of PL with EDTA, as calcium is required by pectate lyases (Sakai et al., 1993). The use of Mayoquest 200 to supply EDTA as chelator at 18 mM concentration, which had been determined from use with Viscozyme, appeared to work adequately with PL. Ariane fibers from Viscozyme-retting were lower in yield and strength (Table 1).

For confirmation of the efficacy of flax retting with PL and EDTA, a variety of flax samples from different sources, including both fiber and linseed types, was enzyme-retted. Results from treatment combinations substantiated and expanded the approach presented in Table 1, showing that PL followed by chelator effectively retted flax from these various sources and gave substantially higher fine-fiber yields and cleaner fibers compared with no retting (data not shown). Furthermore, the improved yield and quality of fibers obtained with PL followed by chelator instead of Bioprep or Mayoquest alone or used in a combined formulation, were confirmed.

Hermes and Omega grown to full seed maturity were enzyme-retted using formulations with PL or Viscozyme in side-by-side tests (Table 2). The Omega sample had rain prior to baling, and substantial weathering had occurred as indicated by darkening of the straw. Hermes, in contrast, was light and showed no effects of weathering prior to enzyme-retting. PL effectively retted both cultivars and resulted in higher fiber yields and fiber with greater tenacity, but Hermes was finer after retting with Viscozyme plus chelator (Table 2).

Scanning electron microscopy showed the structure of flax stems left unretted versus flax stems retted with various components (Fig. 1a–d). The use of PL only for 24 h showed the initial separation of fibers with cuticle still attached (Fig. 1b). The use of chelator was effective in removing the cuticle with or without enzyme (Fig. 1c and d) that is likely caused by the removal of calcium bridging of the non-methoxylated pectin molecules reported to be high in this area (Akin et al., 2004).

By successful retting of flax from several sources with PL followed by chelator, a series of tests was then conducted to optimize the formulation, with fiber yield and remaining shive content as the criteria. The first test of this series was varying the incubation time with chelator for two flax cultivars (Table 3). Fine-fiber yield and cleanliness were better, or tended to be better, in most cases with chelator incubation at 24 h than at 1 or 4 h. Ariane was lower in shive content in all comparisons but had been crimped with fluted rollers,

Table 2
Fiber yield and properties of Hermes and Omega oilseed enzyme-retted with pectate lyase or Viscozyme and chelator

Cultivar	Formulation[a]	Fiber yield (% straw)[b]		Strength (g/tex)	Elongation (%)	Fineness (airflow)
		Flax-PP	Shirley-cleaned			
Hermes	PL; C	36.2 ± 0.8 a	5.9 ± 0.3 bc	36.7 ± 0.9 a	1.9 ± 0.2 a	4.1 ± 0.2 a
	Viscozyme + C	28.8 ± 1.7 b	5.0 ± 0.6 c	21.3 ± 1.8 c	1.4 ± 0.1 b	3.0 ± 0.1 b
Omega	PL; C	35.5 ± 1.0 a	8.4 ± 0.3 a	30.5 ± 0.1 b	2.0 ± 0.1 a	1.1 ± 0.1 c
	Viscozyme + C	33.7 ± 0.6 a	6.3 ± 0 b	20.7 ± 1.5 c	1.1 ± 0.1 c	1.2 ± 0 c

Values within columns with different letters (a–c) differ at $P \leq 0.05$.

[a] Flax fibers were crimped through a nine-roller calender and 150 g of crimped fibers were enzyme-retted in duplicate samples for each variety and each enzyme. Stems then were soaked 2 min in pectate lyase (PL) in 0.1% BioPrep in 0.5 mM sodium borate, pH 8.74 at 50 °C for 1 h. Then, without washing, PL-saturated flax was soaked for 2 min in chelator (C) (Mayoquest 200, 18 mM EDTA), pH 12.0, and incubated at 50–57 °C for a total of 24 h. Viscozyme-retted fibers were soaked in 0.1% Viscozyme L plus chelator (C), pH 5.0, at 40 °C for 24 h.

[b] Yield from stems cleaned through the USDA Flax Fiber Pilot Plant (Flax-PP) and from fiber subsequently passed through the Shirley Analyzer.

which could have affected shive level. Furthermore, the long storage time of Ariane, having been cut in 1999, may have resulted in some decomposition of the plant and contributed to a greater loosening of shive than the recently harvested Hermes. Additional work is required to determine precisely the influence of cultivar or production/harvesting conditions on shive content after enzyme-retting.

Hermes was selected for other tests to optimize the retting formulation. Tests for incubation times with PL, level of PL (without chelator), and levels and incubation times of chelator were evaluated (Table 4). Based on fine-

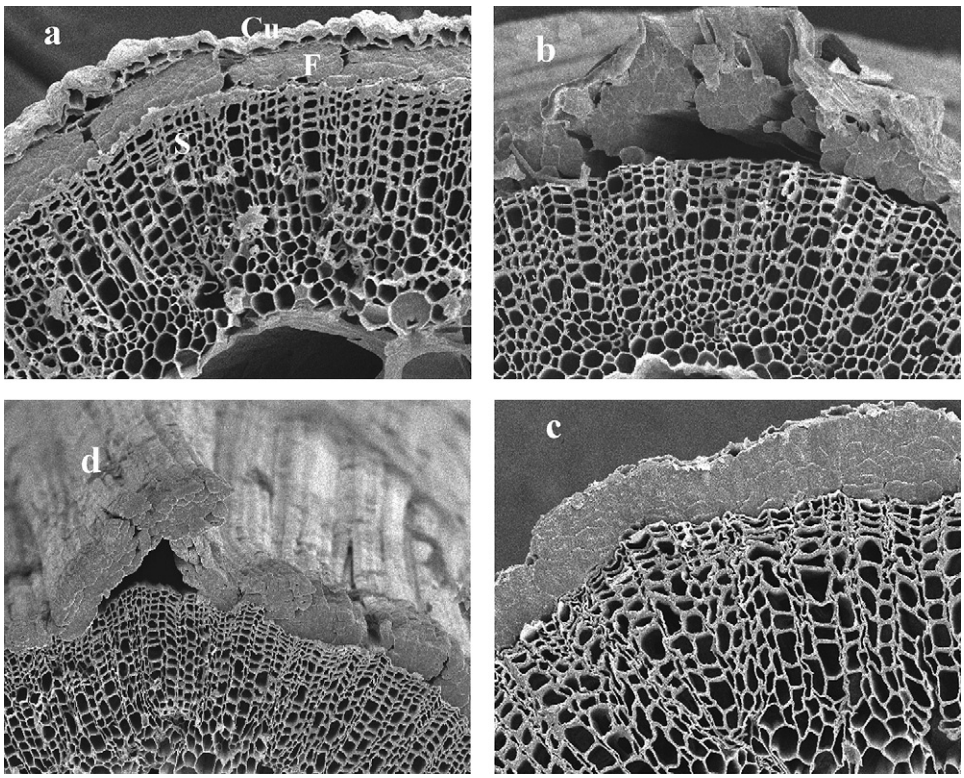

Fig. 1. Scanning electron micrographs of cross sections of Hermes flax stems. (a) Unretted control showing cuticle (Cu) on the outside of the stem, inner lignified core cells that make up the shive (S), and fiber bundles between the epidermis/cuticle layer and shive material (250×). (b) Retted 24 h with PL in 0.5% of commercial enzyme, without chelator, showing disruption of fiber bundles and residual cuticle (200×). (c) Retted 24 h in 18 mM EDTA from commercial product showing loss of cuticle but intact fiber bundles (300×). (d) Retted with PL in 0.5% of commercial enzyme for 1 h and 18 mM EDTA for 23 h showing loss of cuticle and disruption of fiber bundles (150×).

Table 3
Influence of time of incubation on enzyme-retting with pectate lyase

Sample[a]	Treatment[b] (h)	Fiber yield (% straw)			Shive content[c] (%)
		Flax-PP	Shirley-cleaned		
			Flax-PP (%)	Crimped straw (%)	
Ariane	C 1	38.6 ± 2.0 a	6.7 ± 0.7 a	2.7 ± 0.2 a	7.7 ± 1.3 a
	C 4	39.8 ± 3.3 a	8.0 ± 1.5 a	3.2 ± 0.7 a	3.7 ± 1.4 b
	C 24	39.5 ± 1.9 a	13.8 ± 3.4 b	5.4 ± 1.1 b	1.8 ± 0.5 b
Hermes	C 1	42.6 ± 3.5 a	11.5 ± 3.2 a	4.8 ± 0.9 a	8.3 ± 2.2 a
	C 4	41.1 ± 4.9 a	20.7 ± 4.6 ab	8.4 ± 0.9 ab	6.3 ± 0.3 a
	C 24	40.1 ± 0.9 a	30.2 ± 8.5 b	11.7 ± 3.0 b	5.1 ± 0.6 a

Values within columns within cultivars with different letters (a–c) differ at $P \leq 0.05$.
[a] Ariane crimped through fluted rollers and Hermes crimped through nine-roller calender.
[b] Pectate lyase (PL) in 0.1% BioPrep for 1 h followed by chelator (C) (Mayoquest 200 to give 18 mM EDTA) for times listed.
[c] Determined by near infrared spectroscopy for Shirley-cleaned fiber.

fiber yield and % shive content, incubation with PL for 1 h followed by incubation with 18 mM EDTA for 24 h was equal or better than other conditions. Retting effectiveness, however, improved with increased amounts of PL up to 0.5%, which was the highest level tested in this experiment and suggested further increases in enzyme level may improve retting. Furthermore, scanning electron microscopy of retted fibers indicated that PL levels in 5% of the product appeared to remove more contaminants than 0.1% of product (Figs. 2 and 3).

To further optimize the formulation and method for enzyme-retting, Hermes was retted with a range of PL from 0.1% to 5% of product, followed by chelator or combined with chelator in the formulation. The pilot plant cleaned- and Shirley-cleaned fibers are listed in Table 5. The yield of material (i.e., fiber and remaining shive) arising from pilot plant cleaning generally was less with higher enzyme levels and also with enzyme followed by chelator. The pilot plant-cleaned fiber has substantially more shive than the Shirley-cleaned fiber (Table 1). The higher levels of "fiber" with the lower enzyme levels, therefore, arise from fiber plus shive in varying amounts. The PL at 0.5–5.0% of the commercial product followed by chelator resulted in fiber contents ranging from 30.4% to 34.4%. For the Shirley-cleaned fine fiber, PL at 1.0–5.0% of product followed by chelator produced the highest fiber yields and the lowest shive contents of 1.5–2.3%. The PL at 5% of the commercial

Table 4
Fiber yield from tests with different amounts and times of pectate lyase and chelator

Treatment[a]	Shirley-cleaned fiber in[b]		Shive content[c] (%)
	Straw (%)	Flax-PP (%)	
PL in 0.1% BioPrep 1 h; C 23 h	13.2 ± 1.7 a	28.0 ± 2.0 a	7.8 ± 2.0 a
PL in 0.1% BioPrep 3 h; C 21 h	10.1 ± 2.2 bc	22.1 ± 5.8 ab	5.2 ± 1.0 a
PL in 0.1% BioPrep 6 h; C 18 h	10.6 ± 0.9 ab	23.2 ± 1.9 ab	6.7 ± 2.3 a
PL in 0.1% BioPrep 24 h; No C	5.5 ± 0.4 ef	11.2 ± 0.3 e	11.4 ± 2.6 a
PL in 0.2% BioPrep 24 h; No C	6.6 ± 0.8 def	13.5 ± 1.5 de	9.2 ± 2.0 a
PL in 0.5% BioPrep 24 h; No C	8.6 ± 1.2 bcde	18.4 ± 2.6 bcd	6.9 ± 1.6 a
PL in 0.1% BioPrep 1 h; C 8 h	5.2 ± 1.2 f	11.9 ± 2.7 e	5.7 ± 1.2 a
PL in 0.1% BioPrep 1 h; C 24 h	8.1 ± 1.3 bcdef	19.8 ± 4.2 bc	4.3 ± 1.1 a
PL in 0.1% BioPrep 1 h; 4 mM EDTA 24 h	7.4 ± 3.1 cdef	16.0 ± 5.2 cde	7.5 ± 1.4 a
PL in 0.1% BioPrep 1 h; 9 mM EDTA 24 h	7.1 ± 2.7 cdef	16.1 ± 5.0 cde	4.2 ± 2.3 a
PL in 0.1% BioPrep 1 h; 18 mM EDTA 24 h	9.1 ± 1.7 bcd	21.8 ± 2.8 abc	4.2 ± 0.6 a

Values within columns within with different letters (a–e) differ at $P \leq 0.05$.
[a] Pectate lyase (PL) in BioPrep followed by chelator (C) of 18 mM EDTA unless otherwise indicated. Flax is Hermes crimped through nine-roller calender.
[b] Shirley-cleaned fiber as percent of crimped straw and from fiber passed through the USDA Flax Fiber Pilot Plant (Flax-PP).
[c] Determined by near infrared spectroscopy.

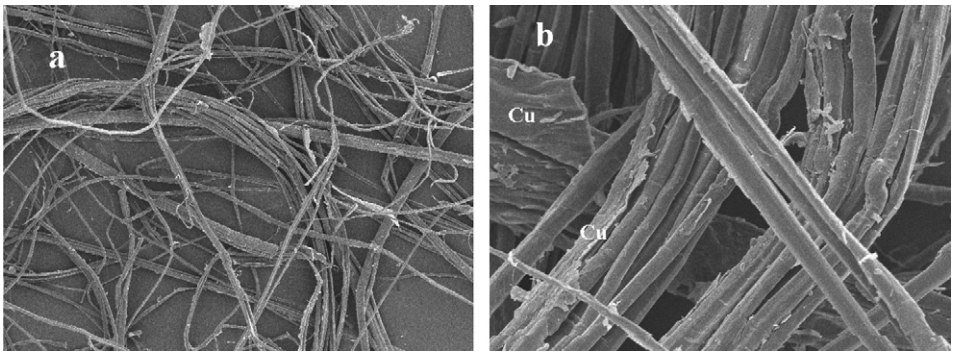

Fig. 2. Retted 24 h with PL in 0.1% of commercial enzyme followed by 18 mM EDTA. (a) Lower magnification showing fiber bundles of various sizes (50×). (b) Enlarged view showing fiber bundles, some of which are comprised of several fibers, with cuticle material (Cu) attached to fibers (300×).

product did not produce higher yields or cleaner fibers than 1–2%. Shirley-cleaned fibers do not represent all the fibers that could be extracted in commercial, cottonizing systems. Therefore, fiber yields from a single pass through the Shirley Analyzer were used only to rank enzyme formulations.

The relative chemical costs to compare formulations, which were derived from fine-fiber yield and amounts of PL and chelator absorbed, are presented in Table 5. These data reflect no adjustments for fiber cleanliness, which was less with lower PL levels. Within this test, costs increased with increasing amounts of PL in the formulation, with lower costs for PL followed by chelator within an enzyme level. While these data provide a simple rating based on laboratory studies, more aggressive cleaning to generate greater fiber yields and reduced chemical costs due to bulk purchasing will give a more accurate accounting of costs.

Shirley-cleaning is used to estimate fine-fiber yield. In one test, waste fibers (coarser fibers and shive) were further cleaned by passing material two more times through the Shirley Analyzer. The additional fiber yields were 2.8–3.9 percentage units greater, with higher yields for chelator following PL treatment compared to chelator combined with PL. The greatest increases of 3.9 percentage units occurred with 5% of the commercial product. The 2.0% product level followed by chelator was further assessed for fine-fiber yield by subsequently passing waste material 11 consecutive times through the Shirley Analyzer. An additional 3.4 percentage units of fiber was obtained by the additional eight passes, giving a total yield of 17.1% Shirley-cleaned fiber from crimped flax stems.

The fibers properties were determined for fibers produced from retting formulations of 0.1–5.0% of the PL product followed with chelator or with enzyme/chelator combined (Table 6). Fiber strength, determined at 6.25 mm gauge distance, did not differ ($P > 0.05$) with any of the enzyme levels even at 5%. These averaged about 33 + 2 g/tex for all formulations. Elongation was low for all enzyme levels and not different ($P > 0.05$). Fiber fineness based on airflow differed ($P \leq 0.05$) with

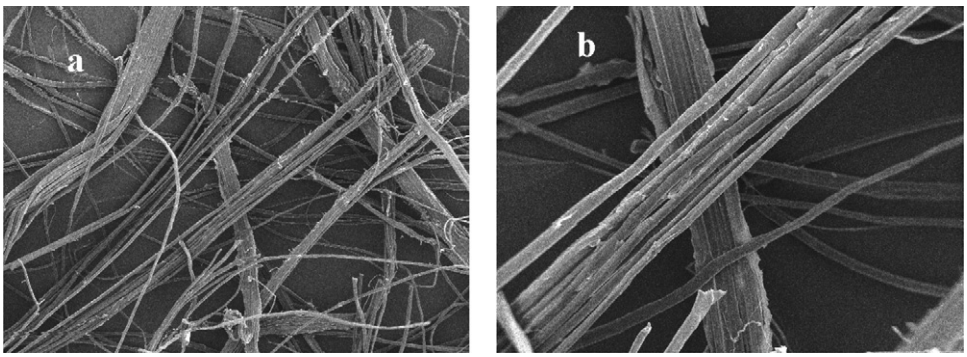

Fig. 3. Retted 24 h with PL in 5.0% of the commercial product followed by 18 mM EDTA. (a) Lower magnification showing considerable separation of bundles to smaller units and fibers (50×). (b) Enlarged view showing fibers and bundles with a clean surface and little non-fibrous material (200×).

Table 5
Yield, cleanliness, and chemical cost of flax fiber retted with various pectase lyase/chelator formulations

Enzyme formulation[a]	Flax-PP fiber		Shirley-cleaned fiber			
	Yield (%)	Relative cost	Yield		Shive content[b] (%)	Relative cost[c]
			Flax-PP (%)	Crimped straw (%)		
0.1%; C	40.9 ± 4.4 bc	1.0	23.9 ± 6.4 cde	10.0 ± 2.9 bcd	5.1 ± 1.9 bc	1.0
0.1% + C	39.9 ± 1.1 bcd	1.3	15.8 ± 0.6 ef	6.3 ± 0.2 ef	4.6 ± 0.9 bc	2.0
0.1% (no C)	49.4 ± 4.1 a	0.4	11.2 ± 0.3 f	5.6 ± 0.4 f	11.4 ± 2.6 a	0.9
0.5%; C	35.1 ± 1.5 efg	2.8	30.7 ± 7.5 bc	10.7 ± 2.5 bcd	2.0 ± 1.1 def	2.1
0.5% + C	36.2 ± 2.6 def	3.5	25.3 ± 5.8 cd	9.3 ± 2.7 bcdef	3.9 ± 1.9 bcd	3.3
0.5% (no C)	42.4 ± 5.2 b	1.9	20.2 ± 2.6 def	8.5 ± 1.0 cdef	5.7 ± 1.7 b	2.1
1.0%; C	34.4 ± 3.0 efg	4.9	34.2 ± 4.7 ab	11.8 ± 2.2 abc	1.7 ± 0.9 ef	3.3
1.0% + C	35.8 ± 3.2 ef	6.0	25.2 ± 2.5 cd	9.0 ± 1.3 bcdef	3.6 ± 1.4 bcdef	5.5
1.0% (no C)	36.7 ± 0.7 cde	4.1	25.9 ± 0.5 cd	9.5 ± 0.3 bcde	3.7 ± 1.5 bcde	3.6
1.5%; C	33.6 ± 2.1 efg	7.1	34.7 ± 5.0 ab	11.8 ± 2.1 abc	1.5 ± 0.2 f	4.7
1.5% + C	32.4 ± 1.2 efg	9.4	25.1 ± 0.9 cd	8.1 ± 0.1 def	3.0 ± 1.1 cdef	8.5
1.5% (no C)	ND	ND	ND	ND	ND	ND
2.0%; C	30.4 ± 2.3 g	10.1	33.2 ± 4.2 ab	10.2 ± 2.0 bcd	2.3 ± 1.2 def	7.1
2.0% + C	31.7 ± 2.3 fg	12.4	23.2 ± 2.1 cde	7.4 ± 1.1 def	2.9 ± 1.3 cdef	12.3
2.0% (no C)	ND	ND	ND	ND	ND	ND
5.0%; C[d]	33.2 ± 1.6 efg	22.4	35.3 ± 6.9 ab	11.7 ± 2.9 abc	1.6 ± 0.5 ef	14.9
5.0% + C[d]	35.8 ± 0.5 ef	26.3	36.8 ± 14.6 a	13.2 ± 5.2 a	2.0 ± 0.4 def	18.9
5.0% (no C)	34.7 ± 2.2 efg	20.6	36.5 ± 5.2 ab	12.7 ± 2.4 ab	2.3 ± 0.8 def	13.1

Values within columns with different letters (a–g) differ at $P \leq 0.05$.

[a] Hermes flax was dried at 55 °C before crimping through the nine-roller calender. %; C = pectate lyase (PL) in BioPrep followed by 1.83% Mayoquest 200 as chelator (C). % + C = PL in BioPrep and C combined in one formulation. Triplicate samples of 150 g were tested.

[b] Determined by near infrared spectroscopy.

[c] Combined costs of PL and C based on amount of chemicals absorbed onto fibers, Shirley-cleaned yield after 1 pass, and costs provided by suppliers.

[d] Mayoquest used at 3.0% (30 mM EDTA) as chelator (C).

enzyme treatments, with finer fibers produced with PL product levels of 1.5–5.0%. Tactile softness of fibers, which was subjectively assessed by two scientists with fiber experience, was better from retting with 1.0% and 2.0% PL of product than with lower enzyme levels (5% PL not rated).

Flax fibers from PL-retting studies, ranging from 0.1% to 5% of the commercial product followed by

Table 6
Properties of Hermes flax fiber enzyme-retted with in various levels of pectate lyase

Enzyme formulation[a]	Strength (g/tex)	Elongation (%)	Fineness (airflow)	Softness (rank)[b]
0.1%; C	34.7 ± 2.1 a	1.5 ± 0.2 a	4.5 ± 0.1 ab	9
0.1% + C	31.8 ± 1.5 a	1.4 ± 0.3 a	4.5 ± 0.1 ab	10
0.5%; C	36.1 ± 3.6 a	1.6 ± 0.2 a	4.5 ± 0.1 ab	8
0.5% + C	33.3 ± 1.4 a	1.4 ± 0.2 a	4.5 ± 0.1 a	7
1%; Ct	32.1 ± 0.7 a	1.6 ± 0.1 a	4.3 ± 0.1 c	2
1% + C	30.6 ± 1.1 a	1.7 ± 0.2 a	4.4 ± 0.1 abc	3
1.5%; Ct	29.8 ± 6.8 a	1.5 ± 0.2 a	4.1 ± 0.1 d	5
1.5% + C	33.2 ± 0.6 a	1.4 ± 0.3 a	4.4 ± 0.1 abc	6
2.0%; C	32.6 ± 0.9 a	1.6 ± 0.3 a	4.1 ± 0.1 d	1
2% + C	31.6 ± 0.6 a	1.5 ± 0.1 a	4.4 ± 0.1 bc	4
5%; C	32.6 ± 1.3 a	1.5 ± 0.3 a	4.2 ± 0.1 de	ND
5% + C	33.9 ± 0.8 a	1.4 ± 0.1 a	4.1 ± 0.1 d	ND
5% (no C)	29.8 ± 3.6 a	1.2 ± 0.2 a	4.2 ± 0.1 de	ND

Values within columns with different letters (a–d) differ at $P \leq 0.05$.

[a] Hermes was grown to full seed maturity at Carrington, ND, in 2004, and used for all tests. Flax was dried at 55 °C before crimping through the nine-roller calender of the USDA Flax Fiber Pilot Plant. %; C = Pectate lyase (PL) in % BioPrep followed by 1.83% Mayoquest 200 as chelator (C). % + C = PL in % BioPrep and C combined in one formulation. Triplicate samples of 150 g were tested.

[b] Subjective ranking from 1 (softest) to 10 (roughest) by two experienced researchers in handling flax and cotton fibers.

18 mM EDTA, were blended with cotton at initial ratios of 1:1. Test yarns were made in a pilot plant ring-spinning system. Loss of fibers after cleaning through the Shirley Analyzer was lowest for the 1%, 2%, and 5% enzyme product (about 16.4% compared with over 20% for other levels). Card losses were less for enzyme-retting levels of 2% and 5% (losses of 6.3% and 6.8%, respectively) enzyme product. Other yarn parameters, such as tenacity and work to rupture, were not linear based on enzyme levels used in retting, possibly reflecting uneven losses of flax in blended yarns. Lower card losses with enzymes levels at 2% and 5% are in line with other data, indicating that these enzyme levels appear to be near optimal for retting under these conditions of testing.

4. Discussion

Viscozyme and other mixed-component pectinase products, whose sources are plant wall-degrading fungi and bacteria, contain cellulases among the other enzymes (Sharma and Van Sumere, 1992a,b). Cellulases preferentially attack fibernodes or kink bands and weaken flax fibers (Akin et al., 2004). Alkaline PL from BioPrep 3000 arises from multiple copies of the native gene for the enzyme inserted into the original *B. lichiniformis* bacterium, thereby providing a high level of pectinase without cellulases. This enzyme, marketed under the trade name BioPrep 3000, was the first commercially available PL and was isolated and produced for its unique ability to degrade the pectin layer between the waxy cuticle and cellulosic fiber of cotton. BioPrep 3000 has performed well as a replacement for alkaline cotton scouring (Durden et al., 2001; Etters et al., 2001).

Pectins serve as complex molecules that bind plant tissues together in both cotton and flax. Pectic substances, determined by response to chemical extractives, are reported to range from 0.7% to 1.2% of cotton fiber dry weight (Wakelyn et al., 1998). Specific determination of pectins in flax is difficult due to several factors including complexity of pectin structure, variations among cultivars, positions on the stem, and fiber quality. Ansari et al. (1990) listed pectin contents in decorticated flax as 20.5%. The content of pectic substances ranges from 26% to 34% of cell walls for various flax cultivars (Brown et al., 1986). Based on hydrolysis with dilute hydrochloric acid followed by ammonium citrate, Bochek et al. (2002) reported a pectin content of flax fibers as 1.6%. It is clear that the amount of non-cellulosic carbohydrates, including pectins, in the bast regions of flax is considerably higher than these constituents in cotton fibers. In flax, pectins bind fibers together in bundles and also bind non-fiber tissues (cuticularized epidermis and woody, core cells) to fiber bundles (Akin et al., 2004; Van Sumere, 1992). Therefore, the level of PL required to ret flax would be expected to be greater than for scouring cotton. Using calculations derived during this study, the level of PL activity for 2% BioPrep can be determined as follows: with approximately 300 ml of liquids of a 2% enzyme solution absorbed onto 150 g straw, 6 ml of enzyme solution would be used. Assuming 1 ml of product weighs about 1 g and Bioprep activity is 3000 APSU units/g, then each gram of crimped flax straw would require about 120 U of activity.

Pectinolytic hydrolyases (e.g., polygalacturonases, and pectate lyases) both attack pectins and separate fibers from other tissues in the flax stems. Earlier work in our laboratory had shown that polygalacturonase alone was sufficient to ret flax fibers, without any added benefit from cellulases, hemicellulases, or other plant cell wall-degrading enzymes (Akin et al., 2004; Evans et al., 2002). Data in the present study further show that alkaline pectate lyase also can separate fibers without other enzymes.

The role of Ca^{2+} chelators, such as EDTA, for improved retting is well known (Sharma, 1987b, 1988; Van Sumere, 1992). Henriksson et al. (1997) showed that the addition of oxalic acid and EDTA with Flaxzyme and other enzyme mixtures facilitated their retting activity. Calcium concentrations in flax tissues, as determined by inductive coupling plasma emission spectroscopy, was more than 5.5-fold higher in the cuticularized epidermis than the bast fibers manually separated from similar bast tissues (Akin et al., 2004). Ca^{2+} chelators likely are effective in retting flax by destabilizing the bridges between Ca^{2+} and polygalacturonic acid, thus leading to disruption and removal of calcium-rich epidermal layers (Sakai et al., 1993; Rihouey et al., 1995; Jauneau et al., 1997). Adamsen et al. (2002) showed that EDTA has substantial Ca^{2+} binding activity even at pH 5, which explains the positive value of EDTA at low pHs optimal for some enzymes such as Viscozyme. The binding capacity of EDTA for Ca^{2+} is, however, considerably greater at alkaline pH (Adamsen et al., 2002), and the use of EDTA at a higher pH should be more efficient in retting. One concern for the strategy of using EDTA with PL is that this enzyme requires Ca^{2+} for activity (Sakai et al., 1993). The suggested method for cotton scouring is to apply the enzyme and later apply the chelator (S. Salmon, Novozymes, personal communication). We confirmed that sequential treatment with enzyme followed by chelator was more effective for retting flax than enzyme alone or enzyme plus chelator in a combined solution.

Fiber strength was maintained at all levels with PL and was significantly greater than for fibers retted with Viscozyme. Therefore, a major objective of enzyme-retting with increased fiber strength was reached with PL followed by EDTA.

The NIR method for shive content is new (ASTM D 7076-05, 2005; Sohn et al., 2004), and in some samples of linseed straw the shive content was over-predicted compared with manual separation of fiber and shive. Some modification and expansion of the NIR model is needed for a more precise prediction of shives from particular flax samples, but values as used herein allowed comparisons of enzyme treatments. This method is the first known to the authors for objectively measuring shive content in flax and was recently approved as an ASTM International test method (ASTM 7076-05). Components may still be present on the fiber surface that impede or interfere with fiber applications (e.g., composites, blending, etc.). Additional work is required to address these questions.

5. Conclusions

In a series of tests ranging up to 5% of the commercial PL product BioPrep with 18 mM EDTA, levels around 2% were determined as optimal with the flax samples used and conditions tested. Fiber yield, fineness, and cleanliness were not improved with higher PL levels. Sequential treatment of PL followed by EDTA was the most effective for retting, but combining both enzyme and EDTA also retted flax. The procedure most effective for producing fine, clean fiber was as follows: (a) saturate crimped flax stems with PL at 2% of the commercial product, (b) incubate for 1 h at 55 °C, (c) without washing, re-soak with 18 mM EDTA at pH 12, (d) continue incubation at 55 °C for about 24 h total time, and (e) wash and dry fiber in preparation for mechanical cleaning.

Both Viscozyme and BioPrep can ret flax, providing fibers with different characteristics. Fiber strength is higher with BioPrep. The attack on fibernodes by cellulases, however, in Viscozyme and other commercial mixtures may be advantageous for easier refining such as pulping. The use of various enzymes for retting, therefore, provides selective properties for different applications.

Acknowledgements

We thank Novozymes North America, Inc., Franklinton, NC, for Viscozyme L; Dexter Chemical LLC, Charlotte, NC, for BioScour 3000, Clavodene CIU and Barapon C-108; and Blaine Schatz of the Carrington Research Extension Center, ND State University for production of linseed. We thank Judy Davis and Nkaku Kisaalita, Russell Research Center, ARS-USDA, Athens, GA, and Mary Ard, School of Veterinary Medicine, University of Georgia, for technical assistance.

Mention of commercial names does not constitute endorsement of a product by USDA but it is used for identification only.

References

Adamsen, A.P.S., Akin, D.E., Rigsby, L.L., 2002. Chelating agents and enzyme retting of flax. Text. Res. J. 72, 296–302.

Akin, D.E., Dodd, R.B., Foulk, J.A., 2005. Pilot plant for processing flax fiber. Ind. Crops Prod. 21, 369–378.

Akin, D.E., Dodd, R.B., Perkins, W., Henriksson, G., Eriksson, K.-E.L., 2000. Spray enzymatic retting: a new method for processing flax fibers. Text. Res. J. 70, 486–494.

Akin, D.E., Foulk, J.A., Dodd, R.B., McAlister III, D.D., 2001. Enzyme-retting of flax and characterization of processed fibers. J. Biotechnol. 89, 193–203.

Akin, D.E., Henriksson, G., Evans, J.D., Adamsen, A.P.S., Foulk, J.A., Dodd, R.B., 2004. Progress in enzyme-retting of flax. J. Nat. Fibers 1, 21–47.

Akin, D.E., Rigsby, L.L., Perkins, W., 1999. Quality properties of flax fibers retted with enzymes. Text. Res. J. 69, 747–753.

Ansari, I.A., East, G.C., Johnson, D.J., 1990. Structure–property relationships in natural cellulosic fibres. Part I. Characterization. J. Text. Inst. 90, 469–480.

Antonov, V., Marek, J., Maixner, V., Vicenec, R., Fischer, H., 2005. How do enzymes contribute to bast fibres industry? In: Proceedings of the 11th Conference for Renewable Resources and Plant Biotechnology, Institute of Natural Fibres, Poznan, Poland, 6–7 June.

ASTM D 1445-95, 2003. Standard test method for breaking strength and elongation of cotton fibers (flat bundle method). Annual Book of Standards, section 7, Textiles. ASTM, West Conshohocken, PA.

ASTM D 7025-04a, 2005. Standard test method for assessing clean flax fiber fineness. Annual Book of Standards, section 7, Textiles. ASTM, West Conshohocken, PA.

ASTM D 7076-05, 2005. Standard test method for the measurement of shives in retted flax. Annual Book of Standards, section 7, Textiles. ASTM, West Conshohocken, PA.

Bochek, A.M., Zabivalova, N.M., Shamolina, I.I., Grishanov, S.A., 2002. Isolation of pectins from flax pedicels and fibers and their characterization. Russ. J. Appl. Chem. 75, 1517–1521.

Brown, A.E., Sharma, H.S.S., Black, D.L.R., 1986. Relationships between pectin content of stems of flax cultivars, fungal cell wall-degrading enzymes and pre-harvest retting. Ann. Appl. Biol. 109, 345–351.

Brühlmann, F., Leupin, M., Erismann, K.H., Fiechter, A., 2000. Enzymatic degumming of ramie bast fibers. J. Biotechnol. 76, 43–50.

Daenekindt, A., 2004. Flax, hemp and allied fibres in the world. Euroflax Newslett. 21 (1), 6–9.

Durden, D.K., Etters, J.N., Sarkar, A.K., Henderson, L.A., Hill, J.E., 2001. Advances in commercial biopreparation of cotton with alkaline pectinase. AATCC Rev. 1 (8), 28–31.

Etters, J.N., Sarkar, A.K., Henderson, L.A., Liu, J., 2001. The influence of biopreparation of cotton with alkaline pectinase on dyeing properties. AATCC Rev. 1 (5), 22–24.

Evans, J.D., Akin, D.E., Foulk, J.A., 2002. Flax-retting by polygalacturonase-containing enzyme mixtures and effects on fiber properties. J. Biotechnol. 97, 223–231.

Henriksson, G., Akin, D.E., Rigsby, L.L., Patel, N., Eriksson, K.-E.L., 1997. Influence of chelating agents and mechanical pretreatment on enzymatic retting of flax. Text. Res. J. 67, 829–836.

Jauneau, A., Quentin, M., Driouich, A., 1997. Micro-heterogeneity of pectins and calcium distribution in the epidermal and cortical parenchyma cell walls of flax hypocotyl. Protoplasma 198, 9–19.

Kozlowski, R., Batog, J., Konczewicz, W., Mackiewicz-Talarczyk, M., Muzyczek, M., Sedelnik, N., Tanska, B., 2005. Latest state-of-art in bast fibers bioprocessing. In: Proceedings of the 11th Conference for Renewable Resources and Plant Biotechnology, Institute of Natural Fibres, Poznan, Poland, 6–7 June.

Lepsch, D., Horal, J.W., 1998. Development of an integrated modular plastic electrical carrier and flax/polypropylene shelf panel for a vehicle rear shelf system. In: Proceedings of the 1998 Society for Automotive Engineering International Congress and Exposition, pp. 87–94, Paper # 980727.

Rihouey, C., Jauneau, A., Cabin-Flaman, A., Demarty, M., Lefebvre, F., Morvan, C., 1995. Calcium and acidic pectin distribution in flax cell walls: evidence for different kinds of linkages in the cell junction and middle lamella of the cortical parenchyma of flax hypocotyl. Plant Physiol. Biochem. 33, 497–508.

Sakai, T., Sakamoto, T., Hallaert, J., Vandamme, E.J., 1993. Pectin, pectinase, and protopectinase: production, properties, and applications. In: Neidleman, S., Laskin, A.I. (Eds.), Advances in Applied Microbiology, 39. Academic Press, San Diego, CA, pp. 213–294.

Sharma, H.S.S., 1987a. Screening of polysaccharide-degrading enzymes for retting flax stem. Int. Biodeterioration 23, 181–186.

Sharma, H.S.S., 1987b. Studies on chemical and enzyme retting of flax on a semi-industrial scale and analysis of the effluents for their physico-chemical components. Int. Biodeterioration 23, 329–342.

Sharma, H.S.S., 1988. Chemical retting of flax using chelating compounds. Ann. Appl. Biol. 113, 159–165.

Sharma, H.S.S., Faughey, G.J., 1999. Comparison of subjective and objective methods to assess flax straw cultivars and fibre quality after dew-retting. Ann. Appl. Biol. 135, 495–501.

Sharma, H.S.S., Van Sumere, C.F. (Eds.), 1992a. The Biology and Processing of Flax. M Publications, Belfast, Northern Ireland, p. 576 pp.

Sharma, H.S.S., Van Sumere, C.F., 1992b. Enzyme treatment of flax. Gen. Eng. Biotechnol. 12, 19–23.

Sohn, M., Barton II, F.E., Morrison III., W.H., Akin, D.E., 2004. Prediction of shive content in pilot plant processed flax by near infrared reflectance spectroscopy. J. Near Infrared Spectros. 12, 251–258.

Van Sumere, C.F., 1992. Retting of flax with special reference to enzyme-retting. In: Sharma, H.S.S., Van Sumere, C.F. (Eds.), The Biology and Processing of Flax. M Publications, Belfast, Northern Ireland, pp. 157–198.

Van Sumere, C.F., Sharma, H.S.S., 1991. Analyses of fine flax fibre produced by enzymatic retting. Aspects Appl. Biol. 28, 15–20.

Wakelyn, P.J., Bertoniere, N.R., French, A.D., Zeronian, S.H., Nevell, T.P., Thibodeaux, D.P., Blanchard, E.J., Calamari, T.A., Triplett, B.A., Bragg, C.K., Welch, C.M., Timpa, J.D., Goynes Jr., W.R., 1998. Cotton fibers. In: Lewin, M., Pearce, E.M. (Eds.), Handbook of Fiber Chemistry. Marcel Dekker, Inc., NY, pp. 577–724.